

Activation of the TGF- β /Smad signaling pathway in focal segmental glomerulosclerosis

Ji HOON KIM, BYOUNG KWON KIM, KYUNG CHUL MOON, HYE KYOUNG HONG, and HYUN SOON LEE

Department of Pathology, Seoul National University College of Medicine, Seoul, Korea

Activation of the TGF- β /Smad signaling pathway in focal segmental glomerulosclerosis.

Background. Although the pathogenetic relevance of transforming growth factor- β (TGF- β) to glomerulosclerosis is well established, it is not known whether a signal transduction cascade of TGF- β is involved in the development of focal segmental glomerulosclerosis (FSGS), nor is it clear how TGF- β 1 is activated during the course of FSGS formation.

Methods. We examined the expression patterns of TGF- β 1, thrombospondin-1 (TSP-1), TGF- β type II receptor (TGF- β IIIR), phosphorylated Smad2/Smad3, and podocyte-specific epitopes [Wilms' tumor protein-1 (WT-1) and glomerular epithelial protein-1 (GLEPP-1)] in 15 renal biopsy specimens with idiopathic FSGS and six renal biopsies with no detectable abnormalities by means of immunohistochemistry. The mRNA expression patterns of TGF- β 1, TGF- β IIIR, and TSP-1 were further evaluated by in situ hybridization in seven biopsies.

Results. In the controls, immunostaining for TGF- β 1, TSP-1, TGF- β IIIR, and phosphorylated Smad2/Smad3 was almost negligible, but an apparent signal for TGF- β 1, TSP-1, and TGF- β IIIR mRNAs was observed in the visceral glomerular epithelial cells (GEC). In the cases of FSGS, the expression levels of TGF- β 1, TSP-1, and TGF- β IIIR proteins and mRNAs and phosphorylated Smad2/Smad3 were significantly increased, particularly in the GEC of the sclerotic segments, wherein WT-1 and GLEPP-1 were not detected.

Conclusion. These results suggest that damage to podocytes may stimulate TGF- β 1, TSP-1, and TGF- β IIIR expression in GEC, thereby activating the Smad signaling pathway and, in so doing, leading to overproduction of the extracellular matrix (ECM). Thus, a signal transduction cascade of the TGF- β /Smad signaling pathway, which is activated in the GEC, appears to be involved in the development of FSGS.

Focal segmental glomerulosclerosis (FSGS) constitutes a pathologic hallmark of progressive glomerular injury and is characterized by segmental collapse of the glomer-

ular tuft with the accumulation of extracellular matrix (ECM). Almost any type of glomerular pathology could be complicated in its chronic course by the presence of FSGS lesions, which would not be able to be morphologically distinguished from primary FSGS [1, 2].

The initial lesion that leads to the development of FSGS seems to be cellular [3–7], and scar formation generally follows [7, 8]. The cellular lesion consists of hyperplastic glomerular epithelial cells (GEC) overlying the sclerotic or collapsed glomerular tufts [3, 4]. Injury to podocytes is the first event to occur in the natural course of FSGS [3, 4], which may result in the phenotypic dysregulation of the podocytes and cell proliferation [7, 9].

Transforming growth factor- β (TGF- β) is a key regulator of ECM [10, 11]. The fibrogenic actions of TGF- β have been implicated in the development of pathologic renal fibrosis [11–13]. In the hyperplastic GEC of FSGS lesions, obtained from cases of IgA nephropathy, the expression of TGF- β 1 protein and mRNA was markedly increased, whereas no significant expression of TGF- β 1 protein was observed in the mesangial proliferative lesions [14]. No study has been done to confirm this in patients with primary FSGS, although segmental intraglomerular staining for TGF- β 1 has been described in some reports [13, 15].

TGF- β is secreted as an inactive precursor, consisting of an active peptide associated with a latency-associated peptide (LAP) [16]. The activation of latent TGF- β can be induced in vitro by the proteolysis of LAP by using denaturing factors [17]. However, its activation in vivo is more complex and is not well understood. Some researchers have suggested that thrombospondin-1 (TSP-1), an ECM protein, is a potent physiologic activator of latent TGF- β [18–20]. The interaction of the KRFFK sequence of TSP-1 with the LAP induces the activation of TGF- β [21]. The presence of TSP-1 has been demonstrated in human crescents [22, 23] and in other early fibrous glomerular scars [22]. So far, no study has sought to determine whether TSP-1 is expressed in the GEC of FSGS lesions, in association with TGF- β .

Activated TGF- β binds to three different types of ser-

Key words: glomerular epithelial cells, thrombospondin-1, TGF- β type II receptor, phosphorylated Smads, extracellular matrix, glomerulosclerosis.

Received for publication January 7, 2003

and in revised form May 5, 2003, and June 23, 2003

Accepted for publication July 7, 2003

© 2003 by the International Society of Nephrology

ine/threonine kinase receptors [24]. Upon TGF- β binding, the constitutively active TGF- β type II receptor (TGF- β IIR) associates with and phosphorylates the type I receptor [25]. The type I receptor kinase then phosphorylates the Smad2 and Smad3 proteins [26, 27]. The phosphorylated Smads form heteromeric complexes with Smad4. These complexes translocate into the nucleus, where they modulate the expression of the target genes [26, 28–32]. Increased expression of the three TGF- β receptors was reported in the lesions of glomerulosclerosis and in crescents [33]. In the glomeruli of *db/db* mice, a genetic model of type 2 diabetes, increased staining for TGF- β IIR protein [34, 35] and Smad3 [35] has been described. As yet, the expression patterns of TGF- β IIR and phosphorylated Smads in the lesions of human FSGS have not been clearly identified.

We hypothesized that damage to podocytes may lead to their phenotypic dysregulation and, consequently, to the activation of the TGF- β /Smad signaling pathway, culminating in the development of FSGS. Thus, this study was designed to demonstrate the presence of TGF- β /Smad signaling proteins in the lesions of FSGS, by evaluating the expression patterns of TGF- β , TSP-1, TGF- β IIR, and phosphorylated Smad2/Smad3.

METHODS

Patients

Fifteen patients diagnosed as having FSGS were selected for this study. Lesions of FSGS were observed in all these patients affecting 6% to 50% [$22 \pm 14\%$ (mean \pm SD)] of the glomeruli. None of these patients had any prior history of diabetes mellitus, cyanotic heart disease, reflux nephropathy, obesity, autoimmune disease, or drug abuse. The control specimens were obtained during kidney donation for transplantation from a 35-year-old man and from five patients with microscopic hematuria, but with no detectable abnormalities. Renal biopsy specimens with more than 10 glomeruli were processed in preparation for light, electron, and immunofluorescence microscopy. In the case of light microscopy, the kidney tissues were fixed overnight in 4% paraformaldehyde at 4°C.

The patients' ages ranged from 12 to 69 years with a mean age of 36 ± 20 years. The male to female ratio was 2:1. Three patients (20%) showed a sign of hypertension, which was defined as having a blood pressure greater than 140/90 mm Hg. Seven patients (47%) had nephrotic-range proteinuria, with a protein excretion of 3.5 g/day or greater at the time of the biopsy. Renal insufficiency, defined as having a serum creatinine level greater than 1.5 mg/dL, was observed in five cases.

Immunohistochemistry

An avidin-biotin-peroxidase procedure (Dakopatts, Glostrup, Denmark) was used for antibody localization.

Paraffin-embedded kidney sections were deparaffinized serially. Subsequently, these sections were baked in a microwave oven for 15 minutes. They were then incubated overnight at 4°C with rabbit anti-TGF- β IIR (Santa Cruz Biotechnologies, Santa Cruz, CA, USA) and anti-human Wilms' tumor protein-1 (WT-1) (Santa Cruz Biotechnologies) at a dilution of 1 in 100. To demonstrate the presence of glomerular epithelial protein-1 (GLEPP-1), sections were incubated with rabbit antihuman GLEPP-1 (a gift from Dr. Roger Wiggins of the University of Michigan) at a dilution of 1 in 1000 for 1½ hours at room temperature. The sections used to demonstrate the presence of TSP-1 were treated with trypsin for 15 minutes and then incubated overnight with goat anti-TSP-1 (Santa Cruz Biotechnologies) at a dilution of 1 in 200. The other sections were baked in a microwave oven for 5 minutes and then incubated with rabbit antiphosphorylated Smad2/Smad3 (Santa Cruz Biotechnologies) at a dilution of 1 in 1000 for 1½ hours at room temperature. To demonstrate the presence of TGF- β 1, deparaffinized sections were treated with 6 mol/L acid-urea for 30 minutes and then incubated overnight at 4°C with rabbit antihuman TGF- β 1 (Santa Cruz Biotechnologies) at a dilution of 1:50. Biotinylated goat antirabbit immunoglobulin or rabbit antigoat immunoglobulin (Dakopatts) were used as a secondary antibody. Endogenous peroxidase activity was quenched with methanol-H₂O₂ solution. A streptavidin-conjugated horseradish peroxidase complex incubation was performed, followed by the addition of diaminobenzidine. The sections were counterstained with Mayer's hematoxylin. Control experiments were performed by omitting the primary antibody or replacing it with the corresponding nonimmune serum.

The expression and distribution patterns of WT-1, GLEPP-1, TGF- β 1, TSP-1, TGF- β IIR, and phosphorylated Smad2/Smad3 in glomeruli were assessed by three pathologists (J.H.K., K.C.M., and H.S.L.) independently in a blinded manner. For each glomerulus showing the lesion of FSGS, a quantitative analysis for TGF- β 1, TSP-1, TGF- β IIR, and phosphorylated Smad2/Smad3 was performed. First, the total number of GEC in the sclerotic segment and nonsclerotic area was separately counted. Next, the number of GEC that stained positive for each antibody was counted in both areas to obtain the percent of podocytes that express the TGF- β /Smad signaling proteins in the sclerotic area and in the nonsclerotic area.

Generation of digoxigenin (DIG)-labeled riboprobes and in situ hybridization histochemistry

A 0.9 kb cDNA for rat TGF- β 1 [36], a 0.6 kb cDNA for human TSP-1 [37], and a 0.5 kb cDNA for human TGF- β IIR [38] were used for this study. These cDNA probes were obtained from the American Type Culture Collection (Rockville, MD, USA). To amplify the cDNA

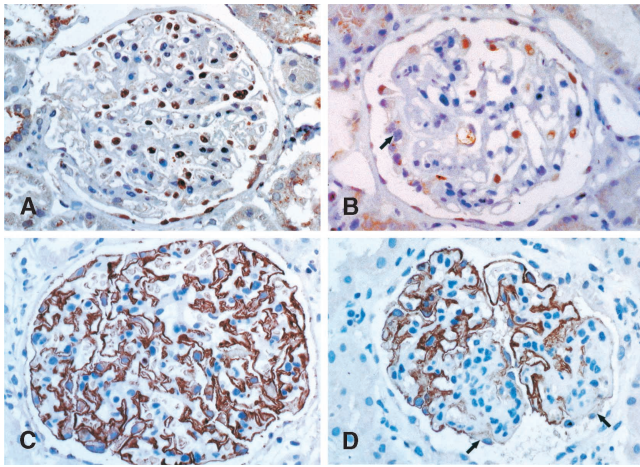


Fig. 1. Immunoperoxidase staining for Wilms' tumor protein-1 (WT-1) (A and B) and glomerular epithelial protein-1 (GLEPP-1) (C and D) in renal biopsy sections. Healthy controls show a nuclear staining for WT-1 in the glomerular epithelial cells (GEC) (A) and a diffuse linear staining for GLEPP-1 along the glomerular capillary wall and the cell membrane of the GEC (C). In the patients with focal segmental glomerulosclerosis (FSGS), the staining for WT-1 (B) or GLEPP-1 (D) is lost in the GEC (arrows) of the sclerotic lesions.

templates, a polymerase chain reaction (PCR) was carried out using T7 and T3 promoters as primers. Two oppositely oriented promoters served to provide two transcripts of the same template, one of which was complementary (antisense), the other being identical (sense) to the target mRNA. DIG-labeled riboprobes were generated using an RNA labeling in vitro transcription kit (DIG RNA Labeling Kit) (Boehringer Mannheim, Germany).

In situ hybridization was performed using paraffin-embedded renal tissues, as described in one of our previous reports [39].

RESULTS

Immunohistochemical studies

WT-1. In the controls, all podocytes expressed WT-1 in the nuclei (Fig. 1A). In the cases of FSGS, WT-1 expression was absent in the GEC covering the sclerotic segments (Fig. 1B).

GLEPP-1. Control biopsies stained with anti-GLEPP-1 showed a diffuse linear staining along the glomerular basement membrane and the cell membrane of the podocytes (Fig. 1C). In the FSGS specimens, there was a loss of GLEPP-1 in the GEC of the sclerotic segments (Fig. 1D). Even in the nonsclerotic glomeruli, immunostaining for GLEPP-1 was segmentally absent.

TGF- β 1. In the controls, there was no staining for TGF- β 1 in the glomeruli (Fig. 2A). In the cases of FSGS, TGF- β 1 was mostly negligible in the nonsclerotic glomeruli, except for a few isolated GEC showing TGF- β 1

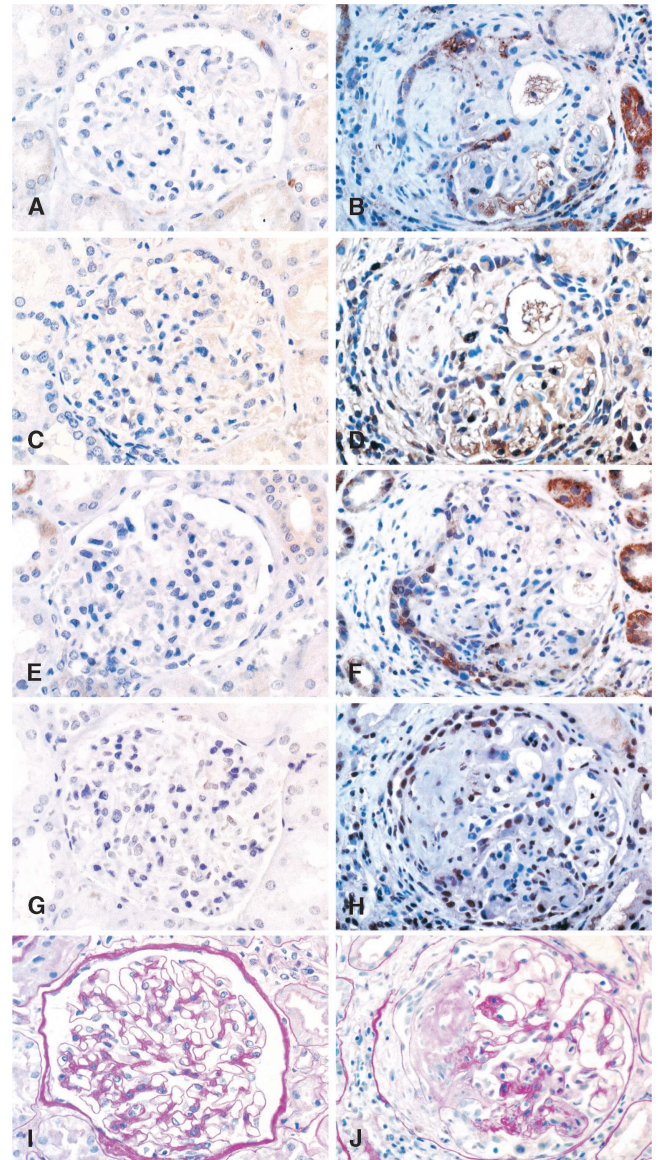


Fig. 2. Immunoperoxidase staining for transforming growth factor- β 1 (TGF- β 1) (A and B), thrombospondin-1 (TSP-1) (C and D), TGF- β type II receptor (TGF- β IIIR) (E and F), and phosphorylated Smad2/Smad3 (G and H) in renal biopsy sections. In healthy controls, there is no staining for TGF- β 1 (A), TSP-1 (C), TGF- β IIIR (E), and phosphorylated Smad2/Smad3 (G). In the cases of FSGS, prominent immunostaining for TGF- β 1 (B), TSP-1 (D), TGF- β IIIR (F), and phosphorylated Smad2/Smad3 (H) is observed in the glomerular epithelial cells (GEC) overlying the sclerotic segments. Control periodic acid-Schiff (PAS)-stained section (I) and PAS-stained serial section showing the location of FSGS lesion (J). (B, D, F, H and J) are serial sections.

immunoreactivity. In the FSGS lesions, moderate to strong immunostaining for TGF- β was apparent in the GEC overlying the sclerotic segments (Table 1) (Fig. 2B). Despite the presence of considerable sclerosis, the viable cells within these lesions retained the ability to overexpress TGF- β 1. However, in the advanced sclerotic lesions with few viable cells, no staining was observed.

Table 1. Expression of transforming growth factor- β (TGF- β)/Smad signaling proteins in podocytes in patients with focal segmental glomerulosclerosis (FSGS)

	Glomeruli examined No.	TGF-β			
TGF-β1		TSP-1	IIR	p-Smad	
Percent-positive podocyte					
FSGS (<i>N</i> = 15)	3.3 ± 1.9 ^a				
Sclerotic A		87 ± 16 ^b	96 ± 6 ^b	87 ± 15 ^b	99 ± 3 ^b
Nonsclerotic A		3 ± 5	8 ± 7	1 ± 2	27 ± 9
Control (<i>N</i> = 6)	5.8 ± 2.1	0	0	0	0

Abbreviations are: A, area; TSP-1, thrombospondin-1; TGF- β IIR, TGF- β type II receptor; p-Smad, phosphorylated Smad2/Smad3. Data are expressed as mean \pm SD.

^aGlomeruli showing the lesion of FSGS

^b $P < 0.05$ vs. nonsclerotic area

TSP-1. TSP-1 was negative in the glomeruli of the control biopsies (Fig. 2C). In the FSGS specimens, only a few isolated GEC exhibited TSP-1 immunostaining in the nonsclerotic glomeruli. TSP staining was distinctively observed in the hyperplastic GEC in the cellular lesions of FSGS (Table 1) (Fig. 2D).

TGF- β IIR. No staining for TGF- β IIR was observed in the glomeruli of the controls (Fig. 2E). In the patients with FSGS, TGF- β IIR expression was negligible in the nonsclerotic glomeruli. In the FSGS lesions, strong TGF- β IIR immunoreactivity was observed in the GEC overlying the sclerotic segments (Table 1) (Fig. 2F).

Phosphorylated Smad2/Smad3. In the controls, there was no immunostaining for phosphorylated Smad2/Smad3 (Fig. 2G). In the cases of FSGS, GEC occasionally exhibited positive nuclear staining for phosphorylated Smad2/Smad3 in the nonsclerotic areas. In the cellular FSGS lesions, intense staining for phosphorylated Smad2/Smad3 was noted in the enlarged nuclei of the hyperplastic GEC (Table 1) (Fig. 2H).

The location of FSGS lesion, which expressed TGF- β , TSP-1, TGF- β IIR, and phosphorylated Smad2/Smad3, was demonstrated in periodic acid-Schiff (PAS)-stained serial section (Fig. 2J) in contrast to control PAS-stained section (Fig. 2I).

In situ hybridization studies

TGF- β 1 mRNA. A weak signal for TGF- β 1 mRNA was shown in several GEC and mesangial cells in the control renal biopsies (Fig. 3A), as described previously [39]. In most of the cases of FSGS, the number of glomerular cells showing an enhanced signal for TGF- β 1 transcripts was increased. All biopsies containing FSGS lesions exhibited intense signals for TGF- β 1 mRNA in the GEC of the sclerotic segments (Fig. 3B). Tubular epithelial cells and vascular endothelial cells frequently showed the message for the TGF- β gene.

TSP-1 mRNA. In the control renal biopsies, the GEC frequently showed a distinctive signal for TSP-1 mRNA

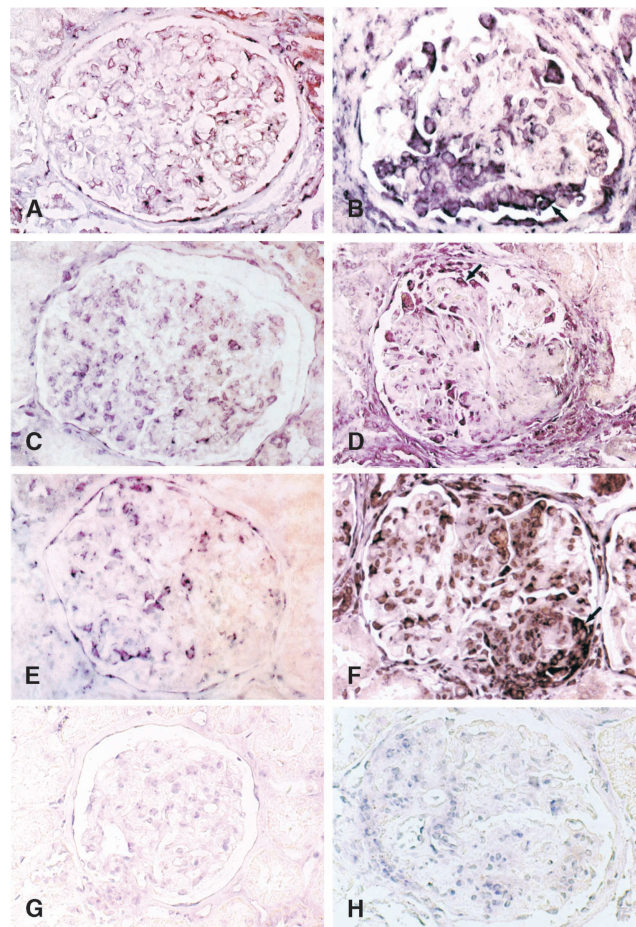


Fig. 3. Detection of transforming growth factor- β 1 (TGF- β 1) (A and B), thrombospondin-1 (TSP-1) (C, D, G, and H), and TGF- β type II receptor (TGF- β IIR) (E and F) mRNAs in renal biopsies of healthy controls (A, C, E, and G) and focal segmental glomerulosclerosis (FSGS) patients (B, D, F, and H). In FSGS, renal biopsy sections hybridized with probes specific for TGF- β 1 (B), TSP-1 (D), and TGF- β IIR (F) show positive signals in the glomerular epithelial cells (GEC) of the sclerotic segments (arrows). Control in situ hybridization with sense TSP-1 riboprobe in renal biopsies of healthy control (G) and FSGS patient (H).

(Fig. 3C). The tubular epithelial cells and vascular endothelial cells occasionally showed the message for the TSP-1 gene. In the FSGS specimens, an intense signal for TSP-1 mRNA was apparent in the GEC overlying the sclerotic segments (Fig. 3D). In the nonsclerotic glomeruli, the number of GEC showing a signal for TSP-1 transcripts was not significantly different from that of the controls.

TGF- β IIR mRNA. In the control renal biopsies, a distinctive signal for TGF- β IIR mRNA was observed in several glomerular cells (Fig. 3E). Tubular epithelial cells and vascular endothelial cells also occasionally showed the message for the TGF- β IIR gene. In the cases of FSGS, a strong signal for TGF- β IIR mRNA was apparent in the hyperplastic GEC overlying the sclerotic segments

(Fig. 3F). In the nonsclerotic glomeruli, the number of glomerular cells showing an enhanced signal for TGF- β IIR transcripts was significantly increased as compared with the controls.

No hybridization was observed in the sections probed with the sense riboprobes for TGF- β 1, TGF- β IIR, and TSP-1 (Fig. 3 G and H).

DISCUSSION

This study demonstrates, to the best of our knowledge for the first time, that the expression of the key components of the TGF- β system, including TGF- β 1, TSP-1, TGF- β IIR, and phosphorylated Smad2/Smad3, is increased in the GEC in the FSGS lesions, wherein WT-1 and GLEPP-1 are not generally detected. These results support the hypothesis that the activation of the TGF- β /Smad signaling cascade in damaged podocytes plays an important role in the pathogenesis of FSGS.

In our patients with FSGS, the expression of TGF- β 1 mRNA and protein was markedly increased in the GEC of the FSGS lesions. However, immunoreactivity for TGF- β 1 in the nonsclerotic glomeruli was mostly negligible, despite the increased expression of its mRNA. These findings are similar to those described in the cellular lesions of FSGS, in patients with IgA nephropathy [14], suggesting that the induction of TGF- β 1 synthesis in GEC could represent a common pathway mediating glomerulosclerosis, regardless of the underlying disease. In partial agreement with our observations, Yamamoto et al [13] reported a segmental intraglomerular staining for TGF- β 1, associated with the matrix, in the case of FSGS.

We also found that TSP-1 protein and mRNA were overexpressed in the cellular lesions of FSGS. Increased TSP-1 expression has been observed in human glomerulopathies associated with early fibrosis [22]. TSP-1 was also particularly prominent in the sclerotic lesions in the remnant kidney model [40], while it was only transiently expressed in the proliferating mesangial cells in the anti-Thy-1.1 nephritis model [41].

TGF- β is usually secreted as a latent complex, and TSP-1 is known to be a major physiologic activator of latent TGF- β [19]. Thus, our demonstration of increased expression of both TSP-1 and TGF- β , in the GEC of the FSGS lesions, suggests that TSP-1 may activate latent TGF- β secreted by GEC. However, the existence of other mechanisms of TGF- β activation, during the development of FSGS, cannot be excluded. Of the various proteolytic factors known to activate latent TGF- β in vitro, plasmin [42–44] or reactive oxygen species [45] could function as an in vivo activator of TGF- β . Latent TGF- β can also be activated via binding to the $\alpha_v\beta_6$ integrin in vivo [46]. Epithelial cells express this particular integrin [47] and, therefore, the above mechanism may also operate on the activation of TGF- β secreted by GEC.

In our patients with FSGS, expression of TGF- β IIR mRNA and protein was also markedly increased in the GEC of the FSGS lesions. In accordance with our findings, Yamamoto et al [33] also observed increased immunostaining for TGF- β receptors in the GEC close to the glomerulosclerotic lesions. TGF- β IIR protein also stained more abundantly in the glomeruli of the *db/db* mice than the controls, particularly in the podocytes [34]. Increased TGF- β IIR expression may allow the podocytes to respond more actively to TGF- β , thus leading to the activation of the TGF- β type I receptor and the Smad pathways.

In the present study, prominent nuclear staining for phosphorylated Smad2/Smad3 was observed in the GEC covering the sclerotic segments. Although diffuse cytoplasmic and nuclear staining for Smad3 has been described in the glomeruli of *db/db* mice [35], this is the first study that demonstrates that the phosphorylated Smads translocated into the nuclei of the GEC in the case of FSGS. The phosphorylated Smads associated with Smad4, which translocated into the nucleus, could activate specific TGF- β 1 target genes, including the ECM component plasminogen activator inhibitor-1, type I collagen, fibronectin, and TGF- β 1 itself [30, 48–51]. Thus, the overproduction of TGF- β 1 protein, and the activation of its downstream signaling in GEC, could lead to continued matrix protein synthesis, resulting in segmental thickening of the glomerular basement membrane. This process may ultimately lead to the segmental collapse of the glomerular capillary tuft and thus to the development of scarring [52, 53].

Podocyte injury is the first event to occur in the natural progression of FSGS [3, 4], resulting in the loss of differentiation markers of podocytes [7, 9]. In rats with reduced renal mass, dedifferentiation and injury of podocytes occurred as a consequence of increased transcapillary passage, and this process seems to be associated with an increased level of TGF- β 1 expression in podocytes [54]. We also observed the disappearance of the podocyte-specific markers, WT-1 and GLEPP-1, in the GEC of FSGS lesions, corroborating the hypothesis that damaged GEC can initiate a cascade of TGF- β signaling.

Earlier studies showed that TGF- β 1 stimulated the expression of type IV collagen and fibronectin in cultured GEC [55]. A recent report demonstrated that TGF- β 1 decreased α 1(IV) and α 5(IV) collagen expression in a mouse podocyte cell line, whereas it increased α 3(IV) collagen expression [34]. The selective analysis of podocytes in vitro was difficult, because only incompletely differentiated podocytes, of questionable cellular origin, were used in most culture studies performed to date [56–59]. Thus, the discordant results on TGF- β 1-induced type IV collagen expression obtained in cultured GEC or the podocyte cell line should be interpreted with caution.

CONCLUSION

We demonstrated the increased expression of TGF- β 1, TSP-1, TGF- β IIIR, and phosphorylated Smad2/Smad3 in the GEC in FSGS lesions, wherein WT-1 and GLEPP-1 were not detected. These results suggest that damage to podocytes may stimulate a signal transduction cascade of the TGF- β /Smad signaling pathway, leading to the overproduction of ECM. Thus, the TGF- β /Smad signaling pathway, which is activated in the GEC, appears to be involved in the development of FSGS.

ACKNOWLEDGMENTS

This work was supported by grants from Seoul National University Hospital. We thank Dr. Roger Wiggins (University of Michigan, Ann Arbor, Michigan) for providing anti-GLEPP-1 antibody.

Reprint requests to Hyun Soon Lee, M.D., Department of Pathology, Seoul National University College of Medicine, Chongno-gu, Yongsong-dong 28, Seoul 110-799, Korea.
E-mail: hyunsoon@plaza.snu.ac.kr

REFERENCES

- CAMERON JS: The enigma of focal segmental glomerulosclerosis. *Kidney Int* 50(Suppl 57):S119–S131, 1996
- WHITWORTH JA, TURNER DR, LEIBOWITZ S, CAMERON JS: Focal segmental sclerosis or scarred focal proliferative glomerulonephritis? *Clin Nephrol* 9:229–235, 1978
- SCHWARTZ MM, LEWIS EJ: Focal segmental glomerulosclerosis: The cellular lesion. *Kidney Int* 28:968–974, 1985
- SCHWARTZ MM, KORBET SM: Primary focal segmental glomerulosclerosis: Pathology, histological variants, and pathogenesis. *Am J Kidney Dis* 22:874–883, 1993
- SCHWARTZ MM, EVANS J, BAIN R, KORBET SM: Focal segmental glomerulosclerosis: Prognostic implications of the cellular lesions. *J Am Soc Nephrol* 10:1900–1907, 1999
- MEYRIER A: Collapsing glomerulopathy: Expanding interest in a shrinking tuft. *Am J Kidney Dis* 33:801–803, 1999
- BARIEY J, BRUNEVAL P, HILL G, et al: Posttransplantation relapse of FSGS is characterized by glomerular epithelial cell transdifferentiation. *J Am Soc Nephrol* 12:261–274, 2001
- KORBET SM, SCHWARTZ MM, LEWIS EJ: Recurrent nephrotic syndrome in renal allografts. *Am J Kidney Dis* 11:270–276, 1988
- BARISONI L, KRIZ W, MUNDEL P, D'AGATI V: The dysregulated podocyte phenotype: A novel concept in the pathogenesis of collapsing idiopathic focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol* 10:51–61, 1999
- ROBERTS AB, HEINE VI, FLANDERS KC, SPORN MB: Transforming growth factor- β . Major role in regulation of extracellular matrix. *Ann N Y Acad Sci* 580:225–232, 1990
- BORDER WA, NOBLE NA: Transforming growth factor β in tissue fibrosis. *N Engl J Med* 331:1286–1292, 1994
- YAMAMOTO T, NOBLE NA, MILLER DE, BORDER WA: Sustained expression of TGF- β 1 underlies development of progressive kidney fibrosis. *Kidney Int* 45:916–927, 1994
- YAMAMOTO T, NOBLE NA, COHEN AH, et al: Expression of transforming growth factor- β isoforms in human glomerular diseases. *Kidney Int* 49:461–469, 1996
- KIM HW, MOON KC, PARK SY, et al: Differential expression of platelet-derived growth factor and transforming growth factor β in relation to progression of IgA nephropathy. *Nephrology* 7:S131–S139, 2002
- YOSHIOKA K, TAKEMURA T, MURAKAMI K, et al: Transforming growth factor- β protein and mRNA in glomeruli in normal and diseased human kidneys. *Lab Invest* 68:154–163, 1993
- MUNGER JS, HARPEL JG, GLEIZES P-E, et al: Latent transforming growth factor- β : Structural features and mechanisms of activation. *Kidney Int* 51:1376–1382, 1997
- BROWN PD, WAKEFIELD LM, LEVINSON AD, et al: Physicochemical activation of recombinant latent transforming growth factor-beta's 1, 2, and 3. *Growth Factors* 3:35–43, 1990
- SHULTZ-CHERRY S, MURPHY-ULLRICH JE: Thrombospondin causes activation of latent transforming growth factor- β secreted by endothelial cells by a novel mechanism. *J Cell Biol* 122:923–932, 1993
- CRAWFORD SE, STELLMACH V, MURPHY-ULLRICH JE, et al: Thrombospondin-1 is a major activator of TGF- β 1 in vivo. *Cell* 93:1159–1170, 1998
- POCZATEK MH, HUGO C, DARLEY-USMAR V, MURPHY-ULLRICH JE: Glucose stimulation of transforming growth factor- β bioactivity in mesangial cells is mediated by thrombospondin-1. *Am J Pathol* 157:1353–1363, 2000
- SHULTZ-CHERRY S, CHEN H, MOSHER DF, et al: Regulation of transforming growth factor- β activation by discrete sequences of thrombospondin 1. *J Biol Chem* 270:7304–7310, 1995
- MCGREGOR B, COLON S, MUTIN M, et al: Thrombospondin in human glomerulopathies. A marker of inflammation and early fibrosis. *Am J Pathol* 144:1281–1287, 1994
- THOMPSON EM, HUGHES J, VAN NOORDEN S, et al: Expression of the multifunctional extracellular matrix protein thrombospondin in crescentic glomerulonephritis. *J Pathol* 178:89–94, 1996
- BLOBE GC, SCHIEMANN WP, LODISH HF: Role of transforming growth factor β in human disease. *N Engl J Med* 342:1350–1358, 2000
- WRANA JL, ATTISANO L, WIESER R, et al: Mechanism of activation of the TGF- β receptor. *Nature* 370:341–347, 1994
- NAKAO A, IMAMURA T, SOUCHELNYTSKYI S, et al: TGF- β receptor-mediated signalling through Smad2, Smad3 and Smad4. *EMBO J* 16:5353–5362, 1997
- MIYAZONO K, TEN DIJKE P, HELDIN C-H: TGF- β signaling by Smad proteins. *Adv Immunol* 75:115–157, 2000
- MACIAS-SILVA M, ABDOLLAH S, HOODLESS PA, et al: MADR2 is a substrate of the TGF β receptor and its phosphorylation is required for nuclear accumulation and signaling. *Cell* 87:1215–1224, 1996
- ZHANG Y, FENG X-H, WU RY, DERYNCK R: Receptor-associated Mad homologues synergize as effectors of the TGF- β response. *Nature* 383:168–172, 1996
- DENNERL S, ITOH S, VIVIEN D, et al: Direct binding of Smad3 and Smad4 to critical TGF β -inducible elements in the promoter of human plasminogen activator inhibitor-type I gene. *EMBO J* 17:3091–3100, 1998
- ZAWEL L, DAI JL, BUCKHAULTS P, et al: Human Smad3 and Smad4 are sequence-specific transcription activators. *Mol Cell* 1:611–617, 1998
- MASSAGUE J, WOTTON D: Transcriptional control by TGF- β /Smad signaling. *EMBO J* 19:1745–1754, 2000
- YAMAMOTO T, WATANABE T, IKEGAYA N, et al: Expression of types I, II, and III TGF- β receptors in human glomerulonephritis. *J Am Soc Nephrol* 9:2253–2261, 1998
- IGLESIAS-DE LA CRUZ MC, ZIYADEH FN, ISONO M, et al: Effects of high glucose and TGF- β 1 on the expression of collagen IV and vascular endothelial growth factor in mouse podocytes. *Kidney Int* 62:901–913, 2002
- HONG SW, ISONO M, CHEN S, et al: Increased glomerular and tubular expression of transforming growth factor- β 1, its type II receptor, and activation of the Smad signaling pathway in the db/db mouse. *Am J Pathol* 158:1653–1663, 2001
- QIAN SQ, KONDAIAH P, ROBERTS AB, SPORN MB: cDNA cloning by PCR of rat transforming growth factor β -1. *Nucleic Acid Res* 18:3059, 1990
- WEINSTAT-SASLOW DL, ZABRENETZKY VS, VANHOUTTE K, et al: Transfection of thrombospondin 1 complementary DNA into a human breast carcinoma cell line reduces primary tumor growth, metastatic potential, and angiogenesis. *Cancer Res* 54:6504–6511, 1994
- BAE HW, GEISER AG, KIM DH, et al: Characterization of the promoter region of the human transforming growth factor- β type II receptor gene. *J Biol Chem* 270:29460–29468, 1995
- KIM TS, KIM JY, HONG HK, LEE HS: mRNA expression of glomerular basement membrane proteins and TGF- β 1 in human membranous nephropathy. *J Pathol* 189:425–430, 1999
- HUGO C, KANG D-H, JOHNSON RJ: Sustained expression of thrombospondin-1 is associated with the development of glomerular and

- tubulointerstitial fibrosis in the remnant kidney model. *Nephron* 90:460–470, 2002
41. HUGO C, PICHLER R, MEEK R, et al: Thrombospondin 1 is expressed by proliferating mesangial cells and is up-regulated by PDGF and bFGF in vivo. *Kidney Int* 48:1846–1856, 1995
 42. LYONS R, GENTRY LE, PURCHIO AF, MOSES HL: Mechanism of activation of latent recombinant transforming growth factor- β 1 by plasmin. *J Cell Biol* 110:1361–1367, 1990
 43. FLAUMENHAFT R, ABE M, MIGNATTI P, et al: Basic fibroblast growth factor-induced activation of latent transforming growth factor β in endothelial cells: Regulation of plasminogen activator activity. *J Cell Biol* 118:901–909, 1992
 44. TAIPALE J, LOHI J, SAARINEN J, et al: Human mast cell chymase and leukocyte elastase release latent transforming growth factor- β 1 from the extracellular matrix of cultured human epithelial and endothelial cells. *J Biol Chem* 270:4689–4696, 1995
 45. BARCELLOS-HOFF MH, DIX TA: Redox-mediated activation of latent transforming growth factor- β 1. *Mol Endocrinol* 10:1077–1083, 1996
 46. MUNGER JS, HUANG X, KAWAKATSU H, et al: The integrin α v β 6 binds and activates latent TGF- β 1: A mechanism for regulating pulmonary inflammation and fibrosis. *Cell* 96:319–328, 1999
 47. BREUSS JM, GILLET N, LU L, et al: Restricted distribution of integrin β 6 mRNA in primate epithelial tissues. *J Histochem Cytochem* 41:1521–1527, 1993
 48. STROSCHEN SL, WANG W, LUO K: Cooperative binding of Smad proteins to two adjacent DNA elements in the plasminogen activator inhibitor-1 promoter mediates transforming growth factor β -induced smad-dependent transcriptional activation. *J Biol Chem* 274:9431–9441, 1999
 49. DATTA PK, BLAKE MC, MOSES HL: Regulation of plasminogen activator inhibitor-1 expression by transforming growth factor- β -induced physical and functional interactions between Smads and Sp1. *J Biol Chem* 275:40014–40019, 2000
 50. MASSAGUE J, CHEN YG: Controlling TGF- β signaling. *Genes Dev* 14:627–644, 2000
 51. VERRECCHIA F, MAUVIEL A: Transforming growth factor-beta signaling through the Smad pathway: Role in extracellular matrix gene expression and regulation. *J Invest Dermatol* 118:211–215, 2002
 52. CHEVALIER J, MASURIER C, LAVAUD S, et al: Approach of cellular mechanisms of glomerulosclerosis in a model of accelerated aging the obese Zucker rat. *C R Seances Soc Biol Fil* 189:987–1007, 1995
 53. BARISONI L, KOPP JB: Modulation of podocyte phenotype in collapsing glomerulopathies. *Microsc Res Tech* 57:254–262, 2002
 54. ABBATE M, ZOJA C, MORIGI M, et al: Transforming growth factor- β 1 is up-regulated by podocytes in response to excess intraglomerular passage of proteins. A central pathway in progressive glomerulosclerosis. *Am J Pathol* 161:2179–2193, 2002
 55. NAKAMURA T, MILLER D, RUOSLAHTI E, BORDER WA: Production of extracellular matrix by glomerular epithelial cells is regulated by transforming growth factor- β 1. *Kidney Int* 41:1213–1221, 1992
 56. HOLTHÖFER H, SAINO K, MIETTINEN A: Rat glomerular cells do not express podocytic markers when cultured in vitro. *Lab Invest* 65: 548–557, 1991
 57. WEINSTEIN T, CAMERON R, KATZ A, SILVERMAN M: Rat glomerular epithelial cells in culture express characteristics of parietal, not visceral, epithelium. *J Am Soc Nephrol* 3:1279–1287, 1992
 58. ORIKASA M, IWAGANA T, TAKAHASHI-IWANAGA H, et al: Macrophagic cells outgrowth from normal rat glomerular culture: Possible metaplastic change from podocytes. *Lab Invest* 75:719–733, 1996
 59. SALEEM MA, O'HARE MJ, REISER J, et al: A conditionally immortalized human podocyte cell line demonstrating nephrin and podocin expression. *J Am Soc Nephrol* 13:630–638, 2002